Correction of Changes in ADP-Induced Platelet Aggregation with Vasopressin Analogue Desglycinamide-Arginine-Vasopressin

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We studied the effect of vasopressin analogue desglycinamide-arginine-vasopressin on changes in platelet hemostasis produced by intragastric administration of Ticlid or clopidogrel (inhibitors of ADP-induced platelet aggregation). Intranasal administration of the peptide under conditions of hemorrhagic diathesis produced a hemostatic effect and normalized some parameters of blood coagulation.

Key Words: *clopidogrel; Ticlid; desglycinamide-arginine-vasopressin; platelet aggregation*

Changes in platelet hemostasis are most prevalent post-hemorrhagic complications [2]. These states can be diagnosed by measuring induced platelet aggregation.

Hemostasis disorders associated with impairment of ADP-induced platelet aggregation can result from a variety of changes, including deficiency of ADP receptors on platelets and absence of ADP in platelet granules [9].

Hemorrhage can be related to changes in ADP binding to some specific receptors [10]. Purine receptors P2Y1 and P2Y12 were extensively studied in this respect. Inhibitors of specific P2Y12 receptors belong to the group of thienopyridine derivatives (ticlopidine, or Ticlid; and clopidogrel, or Plavix). Both preparations initiate a dose-dependent decrease in the number of specific binding sites on platelets and irreversibly inhibit ADP-induced aggregation. Ticlid is used in combination with aspirin to prevent thrombotic complications after coronary stenting [5]. Clopidogrel is indicated for the treatment of vascular atherosclerosis, particularly in patients with contraindications to aspirin therapy [7,8]. Thienopyridine derivatives can be used for experimental modeling of changes in ADP-induced platelet aggregation.

Our previous studies showed that a vasopressin analogue desglycinamide-arginine-vasopressin (DGAVP) is effective in correction of platelet hemostasis disorders. Experimental hemorrhagic diathesis was modeled by administration of aspirin or dipyridamole that modulate prostaglandin metabolism [3].

DGAVP possesses no hormonal activity of vasopressin. This peptide increases the concentrations of factor VIII and von Willebrand factor, but decreases fibrinolytic activity of the blood. We previously found that addition of DGAVP to human blood plasma induces platelet aggregation [4].

Here we studied a hemostatic effect of this peptide under conditions of hemorrhagic diathesis produced by treatment with inhibitors of ADP-induced platelet aggregation (clopidogrel or Ticlid).

MATERIALS AND METHODS

Experiments were performed on outbred albino rats weighing 180-200 g. Antiaggregants Ticlid (70 mg/kg) and clopidogrel (15 mg/kg, Sanofi) were administered intragastrically through a tube 2 h before intranasal treatment with DGAVP in dose of 4 μg/kg (Institute of Extra-Pure Preparations). Administration of DGAVP

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in this dose produces a strong procoagulant effect in intact animals [3]. The animals receiving an equivalent volume of 0.85% NaCl served as the control.

The blood was sampled from the jugular vein before antiaggregant treatment, as well as immediately before and 30 or 60 min after intranasal administration of DGAVP (blood/preservative ratio 9:1). Blood plasma was used to estimate bleeding time (BT) and activated partial thromboplastin time (APTT). ADP-induced platelet aggregation was studied in platelet-rich plasma (1 μM ADP) [1]. The results were subjected to statistical treatment [6].

RESULTS

In series I hemorrhagic state was modeled by intragastric administration of Ticlid. Severe hemorrhage developed 2 h after treatment and manifested in inhibition of ADP-induced platelet aggregation (by 26% compared to the basal level) and increase in BT (by 2.5 times compared to intact animals) and APTT (Table 1, Fig. 1). BT in animals with hemorrhage returned to normal 30 min after intranasal administration of DGAVP. This parameter progressively decreased by the 60th minute after treatment with the peptide (Fig. 1). DGAVP increased ADP-induced platelet aggregation by 10-28% (Table 1).

In series II we showed that clopidogrel is more potent than Ticlid in producing hemorrhage. This antiaggregant increased BT (more than by 3 times compared to the basal level, Fig. 2), completely inhibited ADP-induced platelet aggregation, and increased APTT (Table 2).

TABLE 1. Changes in APTT and ADP-Induced Platelet Aggregation (PA) Produced by Intranasal Administration of DGAVP after Ticlid Treatment (*M*±*m*)

Experimental conditions	APTT, %	PA, %
Ticlid	115.0±2.9	74.0±6.2*
Ticlid and DGAVP		
after 30 min	100.0±1.0	110.0±4.3^{+}
after 60 min	103.0±3.2	128.0±3.2^{++}
Ticlid and 0.85% NaCl		
after 30 min	111.0±2.8	53.0±12.3*
after 60 min	110.0±2.2	56.0±8.9*

Note. Here and in Table 2: biochemical parameters are expressed in percents of the basal level (100%). *p<0.01 compared to the basal level (100%); ^{+}p<0.05 and ^{++}p<0.001 compared to Ticlid.

TABLE 2. Changes in APTT and ADP-Induced Platelet Aggregation (PA) Produced by Intranasal Administration of DGAVP after Clopidogrel Treatment (*M*±*m*)

Experimental conditions	APTT, %	PA, %
Clopidogrel	120.0±2.6	26.0±1.4
Clopidogrel and DGAVP		
after 30 min	107.0±2.6	21.60±0.76*
after 60 min	105.0±3.2	20.0±0.8*
Clopidogrel and 0.85% NaCl		
after 30 min	125.0±1.7	23.60±1.04*
after 60 min	123.0±2.1	21.30±2.18*

Note. *p<0.001 compared to the basal level (100%).

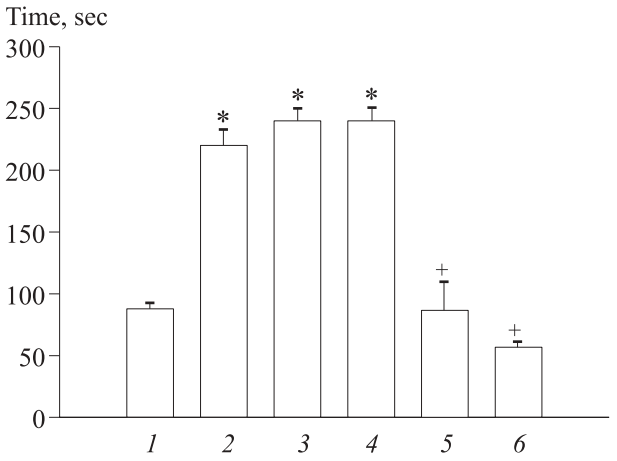

Fig. 1. Changes in the bleeding time produced by intranasal administration of DGAVP after Ticlid treatment: basal level (*1*); Ticlid (*2*); Ticlid+0.85% Nacl, 30th minute (*3*); Ticlid+0.85% Nacl, 60th minute (*4*); Ticlid+DGAVP, 30th minute (5); Ticlid+DGAVP, 60th minute (6). *p<0.001 compared to the basal level; ^{+}p<0.001 compared to Ticlid.

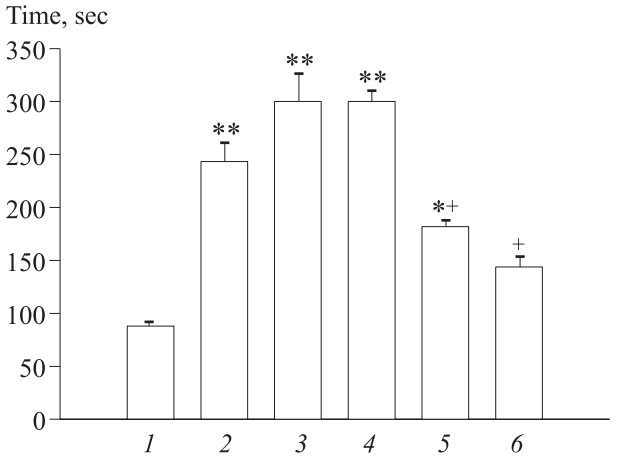

Fig. 2. Changes in the bleeding time produced by intranasal administration of DGAVP after clopidogrel treatment: basal level (*1*); clopidogrel (*2*); clopidogrel+0.85% Nacl, 30th minute (*3*); clopidogrel+0.85% Nacl, 60th minute (*4*); clopidogrel+DGAVP, 30th minute (*5*); clopidogrel+DGAVP, 60th minute (*6*). *p<0.01 and **p<0.001 compared to the basal level; ^{+}p<0.001 compared to clopidogrel.

DGAVP had a less pronounced hemostatic effect in animals treated with clopidogrel. Over 1 h after peptide administration BT remained high, but was below the level observed in control rats (Fig. 2). DGAVP did not modulate clopidogrel blockade of ADP-induced platelet aggregation (Table 2).

Clopidogrel exhibited higher pharmacological activity than Ticlid. The effects of clopidogrel developed more rapidly and persisted over a longer period [5]. These features can explain less significant hemostatic effect of DGAVP in animals with clopidogrel-produced hemorrhagic diathesis. Clopidogrel inhibits tissue factor-induced production of thrombin. These data indicate that clopidogrel possesses not only antiaggregant, but also anticoagulant properties [11]. Our previous studies showed that DGAVP stimulates thrombin production [4]. It can be hypothesized that the hemostatic effect of DGAVP under pathological conditions is associated with activation of thrombin production.

Our results indicate that vasopressin analogue DGAVP can be used as a hemostatic drug for the correction of abnormalities in platelet hemostasis (*e.g.*, disturbances in ADP-induced platelet aggregation). In clinical practice DGAVP can be used not only for normalizing hemostasis during hemorrhage of different genesis, but also for the treatment of bleeding caused by antiaggregant overdose.

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